**The actin associated protein palladin is required for development of normal contractile properties of smooth muscle cells derived from embryoid bodies**

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Running head: role for palladin in smooth muscle contraction
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## Supplementary methods

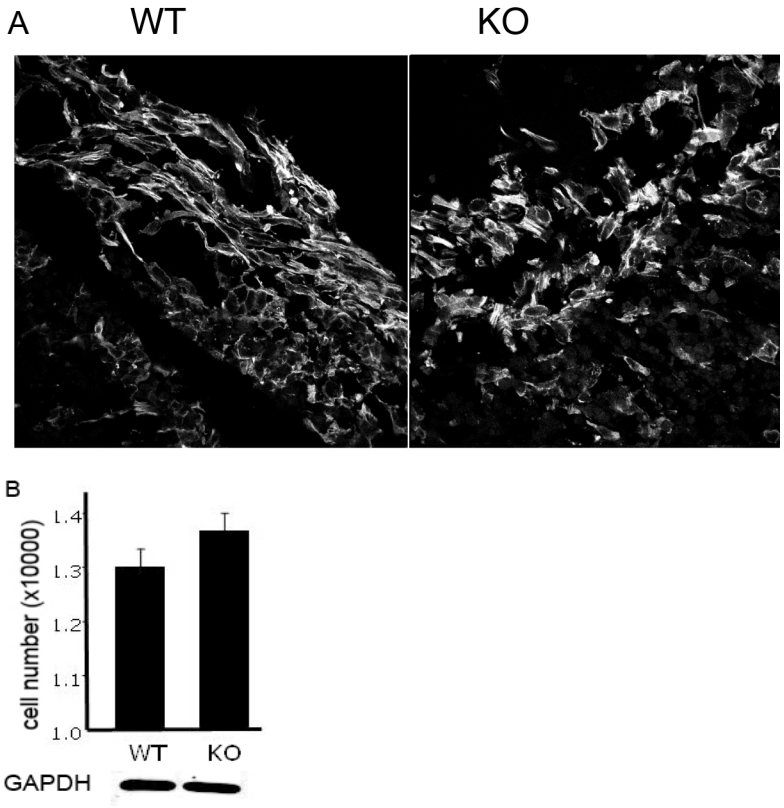
*Rho pull down assay*- The Rho pull down assay was performed according to the manufacturer's instructions (Millipore). Subconfleunt APSCs were serum starved for overnight. Cells were washed three times with cold PBS, and homogenized in lysis buffer. Cell lysates were clarified by centrifugation, and equal volumes of lysates were incubated with Rhotekin RBD-agarose beads (30 µg) at 4°C for 45 minutes. The beads were washed three times with washing buffer. Total and Bound Rho proteins were detected by Western blot analysis using a monoclonal antibody against Rho.

Supplementary figure 1: A, Phalloidin staining showing a similar number of cells in the collagen gels with WT and palladin KO APSCs. WT and KO APSCs were incorporated into collagen gels and cultured for 2 weeks. Strips were cut from the center of the reconstituted fiber and stained with Rhodamine phalloidin. B. Cell counting showing no difference in the number of WT and KO cells in the collagen gels. WT and KO APSCs were incorporated into collagen gels and cultured for 2 weeks. Cells were then dissociated with 0.05% trypsin and 0.1 mg/ml of collagenase, and then counted. Western blotting shows similar contents of GAPDH in WT and KO APSCs incorporated collagen gels (lower panel).

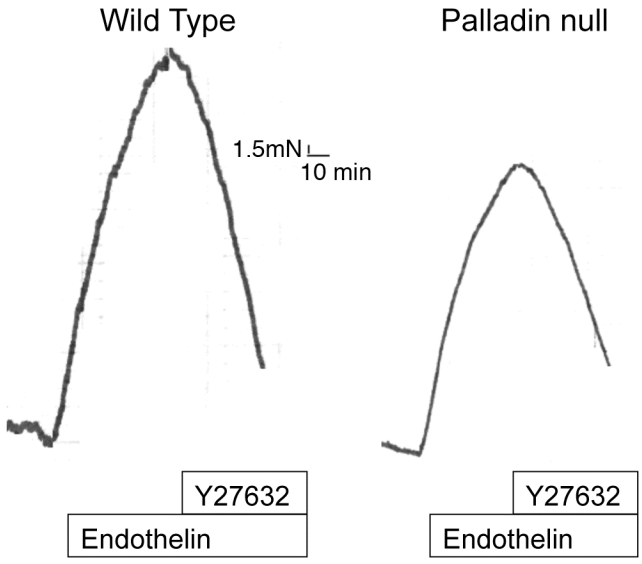
Supplementary figure 2: The ROCK inhibitor Y27632 relaxed endothelin-1 induced tension in WT and palladin KO APSCs. Reconstituted WT and KO APSC fibers were cultured in a collagen 3D-matrix for 2 weeks. Contractions were induced with 1µM endothelin-1 in WT and palladin KO fibers. The generated force is less in the KO fibers. The contractile force can be relaxed with 10 µM of Rho kinase inhibitor, Y27632 in both WT and KO fibers indicating that the $Ca^{2+}$ sensitization pathway is operational. The force traces are representative of three similar experiments.

Supplementary figure 3: Palladin knock out and wild type APSCs showed similar RhoA.GTP activity. Wild type and palladin knock out APSCs were serum starved overnight, and stimulated with 10% serum for 5min. GTP.RhoA was pulled down with Rhotekin conjugated agarose beads. The same amount of protein was loaded for SDS-PAGE and probed with an anti-RhoA monoclonal antibody.

Supplementary figure1




Supplementary figure2



Supplementary figure3